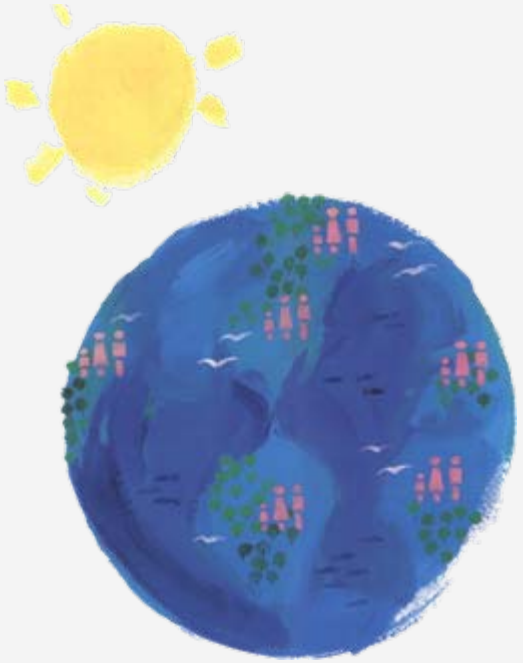
NICEATM

National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods

ICCVAM

Interagency Coordinating Committee on the Validation of Alternative Methods



Availability and Regulatory Acceptance of ICCVAM-Recommended Alternative Test Methods

William Stokes, D.V.M, D.A.C.L.A.M.
RADM, U.S.Public Health Service
Executive Director, ICCVAM
Director, NICEATM

SACATM Meeting
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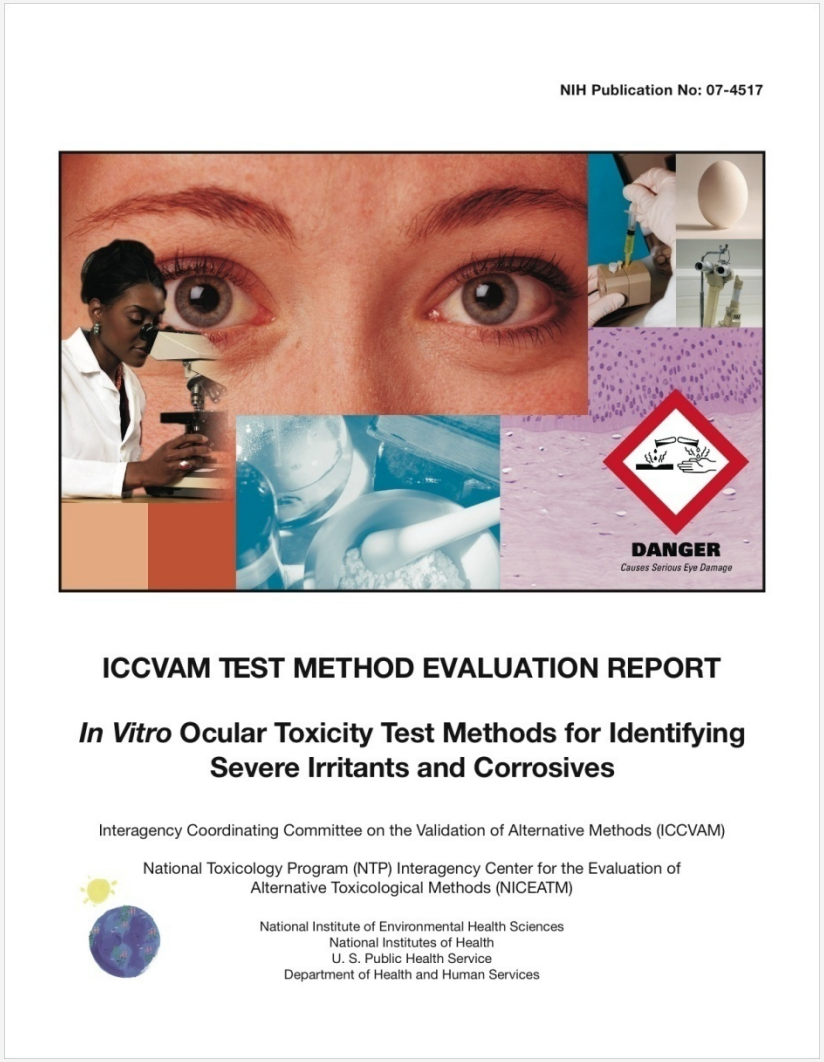
National Toxicology Program

NIEHS

National Institutes of Health

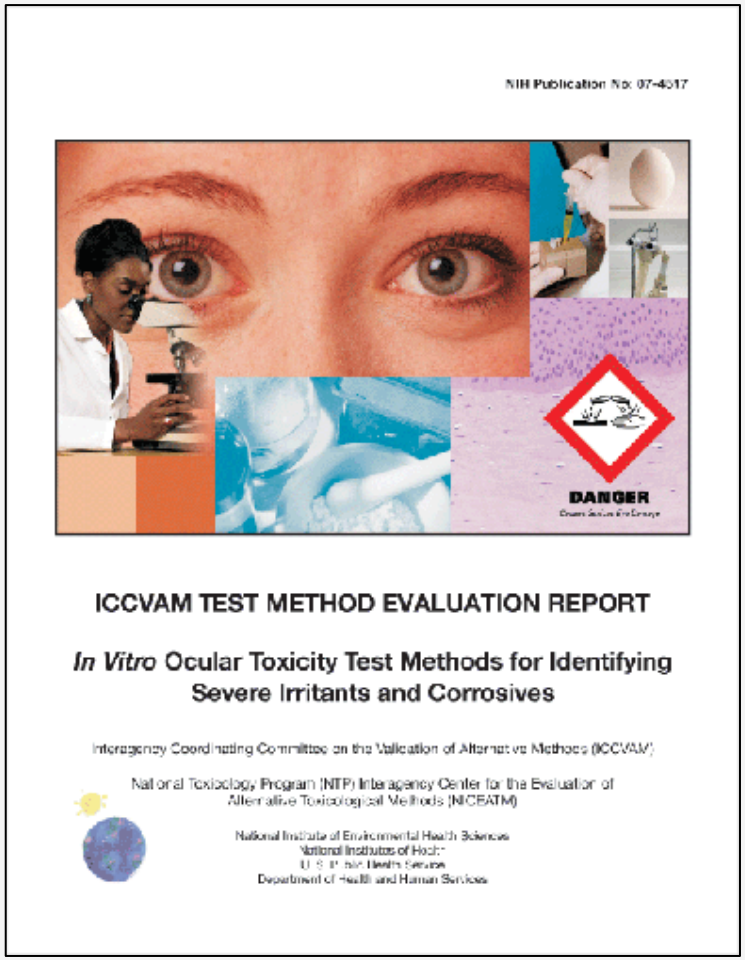
Department of Health & Human Services

Agency Acceptance of ICCVAM Recommendations



- *In Vitro* Ocular Toxicity Test Methods for Identifying Severe Irritants and Corrosives
  - Agency Transmittal
    - Sent to 15 agencies Oct. 26, 2007
    - Responses: April 28, 2008
  - All agencies concurred with the ICCVAM recommendations with the stated limitations and where applicable to their agency
  - These are the first validated in vitro alternative test methods accepted for regulatory use

ICCVAM Recommendations on Four Ocular Safety Testing Methods Accepted by US Agencies



- Bovine Corneal Opacity and Permeability (BCOP) assay
- Isolated Chicken Eye (ICE) assay
- Isolated Rabbit Eye (IRE) assay
- Hen's Egg Test - Chorioallantoic Membrane (HET-CAM) assay

ICCVAM Recommendations: *In Vitro* Ocular Safety Testing Methods

- In accordance with USDA Animal Welfare Act regulations
  - These alternative methods should be considered *before* using rabbits for ocular safety testing, and used where determined appropriate
- Animal Welfare Act responsibilities
  - *Principal Investigators:*
    - *must provide narrative discussion of alternatives consideration in animal study protocols*
  - *Institutional Animal Care and Use Committees:*
    - *must review the consideration of alternatives, and approve animal use*

ICCVAM Recommendations on *In Vitro* Ocular Safety Test Methods

- BCOP and ICE considered useful for regulatory hazard classification testing of some types of substances

- Use in a tiered-testing strategy, where positive substances can be classified as ocular corrosives or severe irritants without the need for animal testing
  - Weight of evidence decisions for positive results
  - Negative substances will require additional testing
    - To detect false negative ocular corrosives or severe irritants
    - To determine if substances may cause moderate or mild irritation
  - Will provide for animal reduction and refinement

- IRE and HET-CAM not considered to *currently* have sufficient performance and/or sufficient data to substantiate use for regulatory hazard classification purposes, but may be useful for other purposes

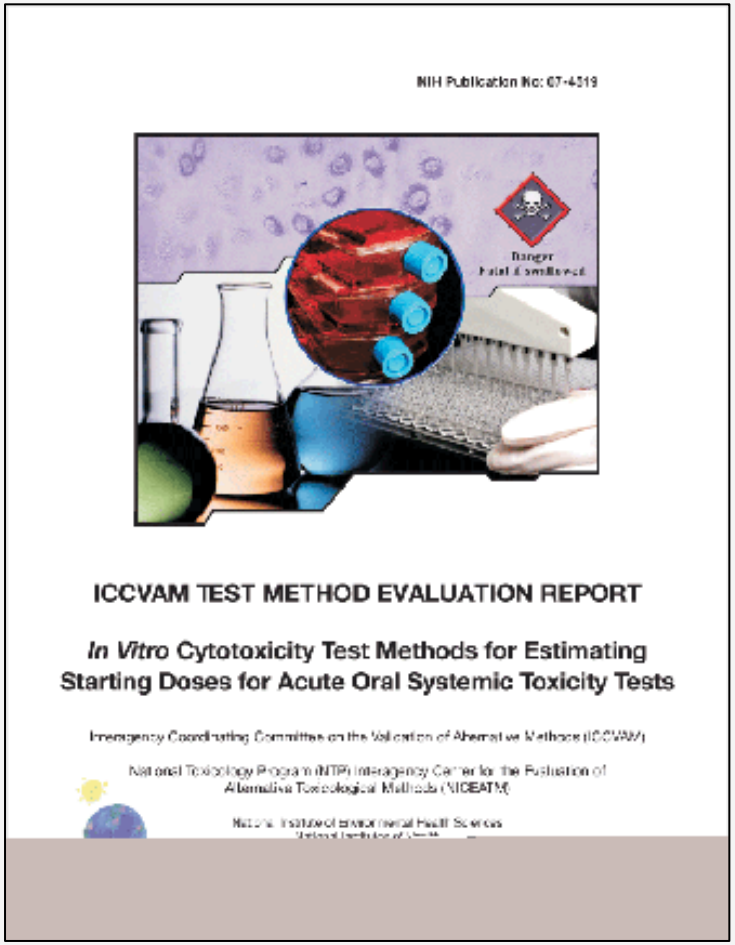
ICCVAM Recommendations: *In Vitro* Ocular Test Methods

- Users encouraged to submit BCOP, ICE, and any in vivo data to NICEATM
  - Data will be used to expand the current validation database, and to reassess usefulness, limitations, and applicability domain for each test method
  - Post-marketing adverse event surveillance and human experience also useful
- Users also encouraged to collect and process tissues for histopathology; forward results to NICEATM for further evaluation
  - Goal is to establish and validate histopathology decision criteria that can be used to improve the accuracy of BCOP and ICE
    - Protocols and Test Guidelines will then be updated to include histopathology
  - Histopathology requested for BCOP, ICE, and rabbit tissues

International Regulatory Acceptance: In Vitro Ocular Safety Methods

- OECD Test Guidelines
  - BCOP and ICE Drafts in development by ICCVAM and NICEATM
    - To include Guidance Document on Histopathology
    - In coordination with ECVAM and JaCVAM
  - Submission to OECD expected in July, 2008
  - OECD has indicated it will expedite processing due to European Commission interest
  - Consideration expected at the National Coordinator Meeting on March 31-April 2, 2009

ICCVAM Recommendations: *In vitro* Methods for Acute Systemic Toxicity

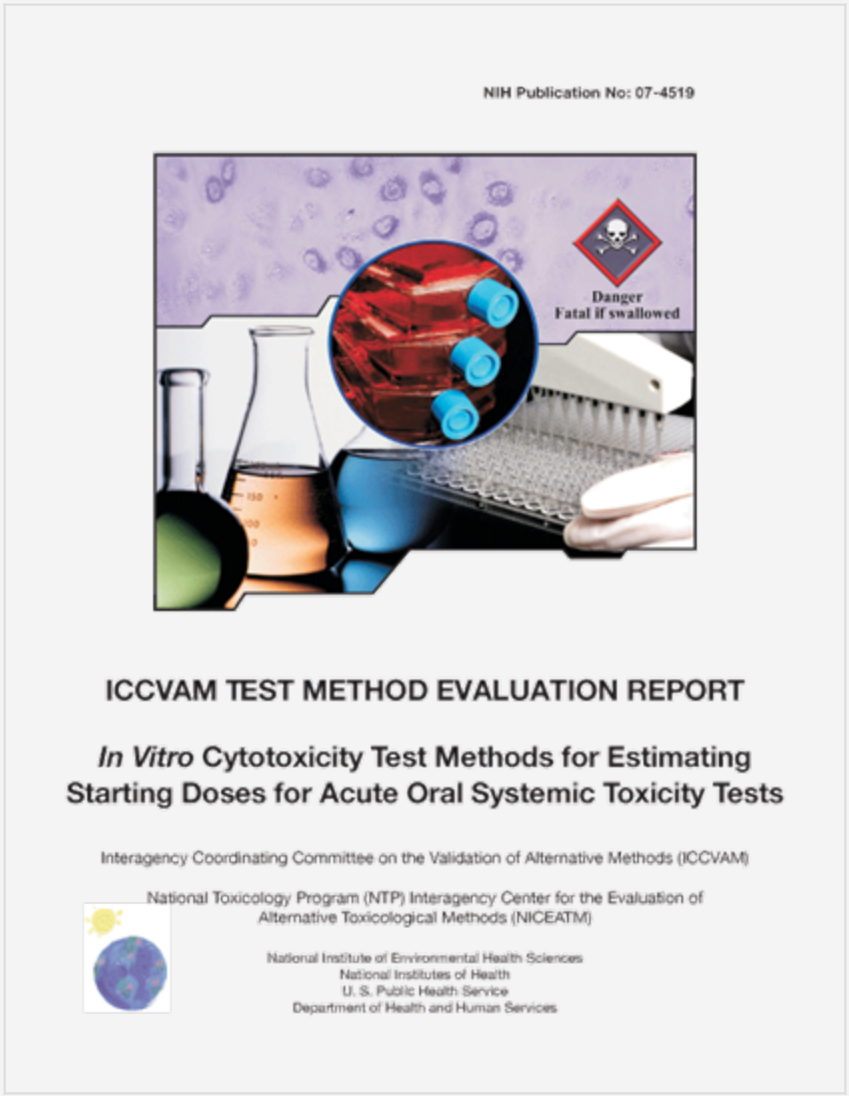


Background

- 2000: International Workshop on In Vitro Methods for Assessing Acute System Toxicity
- 2001: EPA announces the availability of two in vitro basal cytotoxicity test protocols and encourages submission of in vitro data with in vivo data for the HPV program
- 2002-2005: NICEATM-ECVAM In Vitro Validation Study of Two Neutral Red Uptake Cytotoxicity Test Methods
  - To confirm usefulness suggested in 2001 preliminary study
- 2006: Independent scientific peer review
- 2008: Formal transmittal to agencies; public release of final evaluation report

Usefulness has now been confirmed

Status of Agency Consideration of the ICCVAM Recommendations



- *In Vitro* Cytotoxicity Test Methods for Estimating Starting Doses for Actue Oral Systemic Toxicity Tests
  - Agency Transmittal
    - Sent to agencies Feb. 28, 2008
    - Responses due August 28, 2008
  - Two agency responses received as of June 5, 2008
  - Both agencies concur
- Use of these methods does not require regulatory acceptance, as data will not be used for regulatory decisions
- Forwarded to agencies for possible inclusion in test guidelines, and to increase awareness of their availability

ICCVAM Recommendations: *In vitro* Methods for Acute Systemic Toxicity

- May be used in a weight-of-evidence approach to determine starting doses for current acute oral toxicity protocols
  - Up-and-Down Procedure
  - Acute Toxic Class Method

- Should be considered *before* using animals for acute oral toxicity testing, and should be used where determined appropriate

- Where applicable, Principal Investigators should consider these alternatives, and IACUCs should review this consideration and approve animal use, in accordance with:
  - *U.S. Public Health Service Policy on Humane Care and Use of Laboratory Animals*
  - *U .S. Government Principles for Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training*

ICCVAM Recommendations: *In vitro* Methods for Acute Systemic Toxicity

Reduction

- For some types of substances, weight-of-evidence approach will reduce the number of animals needed
  - Up to 50 percent reduction in animal use for “non-toxic” unclassified substances(LD50 >5000mg) compared to starting with default dose
    - 3 vs. 6 animals
  - Estimated that over 50% of all substances tested are non-toxic

Refinement

- In some testing situations, the approach may also reduce the numbers of animals that die or need to be humanely killed

  Applicable to more toxic substances (LD50<175mg/kg)

ICCVAM Recommendations: *In vitro* Methods for Acute Systemic Toxicity

Limitations

- Currently not sufficiently accurate to predict acute oral toxicity for the purpose of regulatory hazard classification categories

- Will likely overestimate starting doses for substances with certain toxic mechanisms not expected to be active in 3T3 or NHK cells (e.g., those that are neurotoxic or cardiotoxic)

  Therefore may not be appropriate for estimating starting doses for such substances

ICCVAM Recommendations: *In vitro* Methods for Acute Systemic Toxicity

- To further characterize the usefulness and limitations for these test methods, *and* to advance the use of *in vitro* methods for assessing acute oral toxicity for regulatory hazard classification purposes:
  - Additional comparative *in vitro* basal cytotoxicity data should be collected when rat acute oral toxicity testing is conducted
    - However, *in vivo* testing should not be conducted solely to collect this data
  - Both in vitro and in vivo data should be submitted to NICEATM for further analysis of the validity of these methods

Questions for SACATM

How can we:

1. Increase awareness of these methods?

2. Encourage their consideration and use?

3. Encourage data submission and optional activities (e.g. histopathology) to aid in increasing usefulness of these methods?